10th DREAM Research Symposium

Presentations:

1. Patience Obi:

   Chronic exercise modifies skeletal muscle-derived extracellular vesicles that in turn transmit exercise-associated metabolic adaptations in a cell-specific manner

2. Benjamin Bydak:

   Extracellular vesicles transmit senescence in human skeletal muscle cells

3. Manuel Sebastian:

   Does the HNF- 1αG319S variant confer a metabolic advantage to a traditional First Nations lifestyle but promote youth onset type 2 diabetes under modern dietary conditions?

4. Lucas Mosienko:

   Predictors of Quality of Life in Youth Living with Type 2 Diabetes

5. Taylor Morriseau:

   Aligning Dietary Intake to nutrient metabolism in experimental models of the HNF- 1αG319S variant

6. Nicole Brunton:

   Ideal cardiovascular health metrics in youth living with Type 2 Diabetes

7. Hannah Steiman De Visser:

   Early Life Adversity and Obesity at 18: A perspective Cohort Study

Chronic exercise modifies skeletal muscle-derived extracellular vesicles that in turn transmit exercise-associated metabolic adaptations in a cell-specific manner

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Introduction: Regular exercise evokes positive systemic benefits such as an increase in mitochondrial biogenesis (MitoB) and potential reduction in tumor growth. Extracellular vesicles (EVs) are small lipid membrane-bound structures that enclose biological cargo, and constitute an essential method of cellular communication. Skeletal muscle releases EVs during exercise, but their characteristics and effects are poorly understood. We hypothesize that exercise alters muscle-EV biophysical profile, and that these muscle-EVs can transmit the pro-metabolic effects of exercise.

Methods: C2C12 myoblasts were differentiated into myotubes, and electrically paced (3h/day x 4days @14V, C-PACE EM, IonOptix) to mimic chronic exercise *in vitro*. EVs were isolated from conditioned media from control and stimulated myotubes using differential ultracentrifugation. Isolated EVs were characterized biophysically (size, zeta potential, yield and protein markers). Myoblasts and Lewis lung carcinoma (LLC) cells were treated with control or stimulated EVs daily for 4 days, and analyzed for changes in MitoB and cell count.

Results: Average EV size was 26% smaller in stimulated *vs*. control EVs ($p<0.05$). Stimulated EVs were enriched with 100-150nm EVs, while control EVs were enriched between 200-250nm ($p<0.05$). Zeta potential and expression of exosomal markers, CD81 and HSP70 were increased in stimulated *vs*. control EVs ($p<0.05$, N=8). EV protein yield remained unchanged between groups. Myoblasts treated with stimulated EVs had increased markers of MitoB: mitochondrial mass (6%), electron transport chain subunit CIV-MTCO1 protein expression (38%), and cytochrome *c* oxidase activity (56%) *vs.* myoblasts treated with control EVs ($p<0.05$, N=6). LLC cells treated with stimulated EVs had lower cell count and viability *vs.* cells treated with control EVs ($p<0.05$, N=6), but no effect on metabolism unlike myoblasts.

Conclusions: Our data show that stimulation evoked the release of more stable, and smaller sized muscle-EVs that increased MitoB in non-exercised muscle cells and reduced cell count/viability in lung cancer cells.

Extracellular vesicles transmit senescence in human skeletal muscle cells

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Introduction: Frailty is characterized by a decrease in functional capacity resulting in increased dependence with age. Epigenetic age (eAge) approximates biological aging, the rate of metabolic and physiological decline with time in cells. We characterized the biophysical characteristics of extracellular vesicles (EVs) with frailty status. We also determined the effects of robust EVs from epigenetically young, and frail EVs from epigenetically old participants, on young and old human skeletal muscle cells.

Methods: Plasma from robust and frail women as determined by the frailty index (64±5.9 years, WARM hearts study (REB H2019:063)) was used for EV isolation through size exclusion chromatography (N=23/group). Participants were matched for age, gender, sex, ethnicity, smoking status, and personal income, and EVs from robust (rEVs) and frail (fEVs) participants characterized. Participants were ranked by eAge and divided into robust/youngest and frail/oldest. Young (19 yrs) and old (92 yrs) human muscle cells were treated with 5% plasma or r/fEVs from participants.

Results: fEVs had 22% more protein than rEVs ($p<0.05$, N=23). HSP70 was 61% higher and ApoA1 119% lower in fEVs *vs*. rEVs ($p<0.05$, N=7-8). Size and zeta potential remained unchanged between groups. fEV treatment of young cells showed 73% increase in senescence as measured by ß-galactosidase staining ($p<0.01$) and a 24% reduction in the viability of old cells ($p<0.05$, N=6-7). rEVs decreased senescence by 39% in old cells ($p<0.05$, N=6-7). Mitochondrial mass was unchanged in young and old cells with rEV or fEV treatment.

Conclusion: Our data show that fEVs have more protein, are enriched with HSP70 but contain less ApoA1, and induced senescence in young cells *vs*. rEVs. In contrast, rEVs rescued senescence in old cells. Neither r/fEVs had any effect on mitochondrial mass. Identification of the biomolecular cargo in EVs will help acquire mechanistic insight into their effects on the cellular indices of aging.

Does the HNF-1aG319S variant confer a metabolic advantage to a traditional First Nations lifestyle but promote youth onset type 2 diabetes under modern dietary conditions?

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BACKGROUND: Genetic testing in Anishininew communities in central Canada led to the discovery of the HNF-1aG319S variant, which may contribute to youth-onset T2D. HNF-1a is a transcription factor that controls glucose and lipid regulation in the pancreas and liver; however, it is unclear how the G319S variant influences these pathways. Given the metabolic demand associated with traditional lifestyle practices in Northern Manitoba, the G319S variant may instead confer an advantage to prolonged fasting and/or very low carbohydrate intake. Here, we examine the impact of prolonged fasting on glucose and lipid metabolism in G/S and S/S expressing mice compared to control (G/G).

METHODS: 3-month-old male and female mice were divided into 2 groups: non-fasted (NF) and fasted for 24hr to assess blood glucose (BG) and ketones (BK). Livers were collected for gene expression, triglyceride and glycogen contents. Islets were isolated to assess insulin secretion capacity.

RESULTS: In male mice, the G319S variant led to decreased blood glucose and increased trend towards ketone production. This was evidenced by higher trends of BK in females and increased ketogenic gene expression in both males and females. Insulin secretion was impaired in G/S mice compared to G/G mice, which maintained insulin secretion capacity.

CONCLUSIONS: The G319S variant appears to alter pancreas and liver metabolism to prevent depletion of insulin during fasting and promote ketogenesis. This data suggest that the G319S variant may confer a metabolic advantage during prolonged fasting.

Predictors of Quality of Life in Youth Living with Type 2 Diabetes

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Background

Prevalence of type 2 diabetes (T2D) is increasing in youth (<18 years). The biopsychosocial impact of T2D on quality of life (QOL) is understudied. We hypothesized that QOL is lower among youth with T2D compared to peers without T2D and is associated with biopsychosocial stressors.

Methods

Data from the Improving Renal Complications in Adolescents with type 2 diabetes through Research (iCARE) study was used to test these hypotheses (n= 331 youth with T2D and 137 peers without T2D). The primary outcome, QOL was measured with the Pediatric Quality of Life Inventory™ (PedsQL). Psychosocial covariates included food security (Household Food security survey module), income quintile, perceived stress (PSS-14) and mental distress (Kessler-6 scale). Biological covariates included age, sex, BMIzscore, HbA1C, estimated GFR and albuminuria (ACR). Regression analyses were used to evaluate differences in QOL and relationships with biopsychosocial covariates between groups and within the T2D group.

Main Findings

Mean age (14.95 vs 14.93 yrs), sex (43.8% vs 35% male), median BMI z scores (2.54 vs 2.10) and indigenous ethnicity (89.1 %vs 81.2%) did not differ between groups. Mean duration of diabetes was 2.3 ± 2.0 yrs. Youth with T2D had significantly lower PedsQL scores (67.01 ± 14.75) compared to controls (71.71 ±16.15), p=0.039. However, diabetes status alone was not independently associated with decreased QOL in multivariate analysis. Mental distress (β = -1.43; P<0.01) and severe food insecurity (β = -6.26; p=0.037) were negatively associated with QOL within the youth with T2D. These associations were significant in both the psychological and physical domains of QOL.

Conclusions

QOL is lower in youth with T2D compared to their peers. However, after controlling for covariates this difference was attenuated. Among youth with T2D, predictors of decreased QoL included mental distress and food insecurity, suggesting areas for further support for these youth.

Aligning dietary intake to nutrient metabolism in experimental models of the HNF-1αG319S variant

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Background: 40% of Indigenous youth with type 2 diabetes (T2D) in Manitoba carry a variant in the HNF-1α gene (HNF-1αG319S). The G319S variant is thought to drive pancreatic β-cell dysfunction; however, youth-onset T2D is a relatively recent phenomenon. We hypothesize the G319S variant impairs β-cell insulin secretion when exposed to modern dietary carbohydrate stress but is protective in the context of traditional off-the-land foods rich in fat and protein.

Methods: CRISPR/Cas9 was used to knock-in the G>A.955 substitution into MIN6 β-cells (G319S-MIN6) and C57/BL6 mice. Mice were weaned onto (1) standard rodent chow, (2) a high-fat, low-carbohydrate (HFLC) diet reflecting off-the-land foods, or (3) a high-fat, high-carbohydrate (HFHC) diet reflecting present-day diet patterns. β-cell function was assessed by glucose-stimulated insulin secretion (GSIS) and oxygen consumption rates for glucose or palmitate oxidation.

Results: Chronic exposure to palmitate impaired glucose-stimulated insulin secretion in wild-type MIN6 β-cells; however, G319S-MIN6 cells were protected via elevated fatty-acid β-oxidation (1.5-fold). Given this propensity for fatty-acid metabolism, G319S-expressing mice retained both glucose tolerance and islet GSIS when fed a HFLC diet that otherwise impaired wild-type mice at 12-weeks-of-age. Conversely, a present-day HFHC diet elevated weight gain and impaired islet GSIS in G319S-expressing mice up to 24-weeks-of-age.

Conclusion: The G319S variant appears to shift β-cell metabolism towards fatty-acid oxidation. To align nutritional intake with this metabolic shift, the consumption of a HFLC diet appears to normalize insulin secretion and glucose tolerance in G319S carriers, although studies to assess long-term effects are underway. Conversely, the HFHC diet worsens metabolic outcomes across all genotypes. These studies may inform nutritional interventions for children with T2D while ultimately supporting community efforts to access off-the-land foods.

Ideal cardiovascular health metrics in youth living with type 2 diabetes

Background: The American Heart Association's (AHA) Life's Simple 7 uses 7 risk factors to quantify cardiovascular (CV) health. As far as we know, no one has applied this concept to youth living with type 2 diabetes (T2D).
Objective: To assess the prevalence of ideal, intermediate, and poor levels of CV health in a group of youth living with T2D.
Methods: This study was done using data from the Improving Renal Complications in Adolescents with T2D through Research (iCARE) prospective cohort. We modified AHA's Ideal CV health targets to include 6 categories that work within the context of T2D. These included blood pressure, cholesterol, glycemic control, diet, physical activity, and smoking. We constructed a CV health score from 0 to 6 by treating each target as a binary variable in which participants received 1 point for having reached the target and 0 points for not having reached it. If participants met 5 or more targets their cardiovascular health status was considered "ideal", "intermediate" if they met 3-4 targets, and they were considered to have "poor" CV health if they met 2 or fewer of the 6 targets.
Results: Only 58 participants had data across all 6 categories. Due to changes in the study protocol over time there is some systematic missingness as not all the same data were collected throughout the study protocol. Among the 58 with complete data, only 7% were considered to have "ideal" cardiovascular health. When we included every participant in the score, despite having missing data on some metrics, 17% of participants were considered to have "ideal" cardiovascular health.
Conclusion: There may be a larger social context to consider when applying these metrics to youth living with T2D. Behaviour change might not be the priority when there are social and structural determinants of health to consider.

299/300 words

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Early Life Adversity and Obesity at 18: A Prospective Cohort Study

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Introduction: Nearly 1 in every 3 adolescents in Canada lives with overweight or obesity. Exposure to adverse childhood experiences (ACEs) increases an adolescent's risk of obesity, however the factors that mediate this association are unclear. We hypothesized that exposure to ACEs before 9 yrs old would be associated with higher BMI at 18 yrs and that this association would be partially mediated by psychological-emotional factors.

Methods: We studied 6942 adolescents that provided data at 9, 13 and 18 yrs of age in the Growing up in Ireland cohort study. The main exposures were 14 ACEs, 4 of which were included in the original ACEs study. The primary outcome was Body Mass Index measured objectively at 18 yrs. The mediators were behavioural difficulties (strengths and difficulties questionnaire (SDQ)) and self-concept (Piers Harris self-concept scale (PH)).

Results: Among the 6942 adolescents with data from all 3 visits, 49% were female and 26.5% were overweight/obese at 18 years. At all three ages BMI was significantly higher in those exposed to an ACE, compared to those were not. In the fully adjusted regression models at 13 yrs of age, ACEs were associated with a 2-point increase (95% CI: 1.8 to 2.6) to the total SDQ score and a 1-point decrease (95% CI: -1.6 to -0.5) in the PH score, but not associated with diet or exercise. Higher behavioural difficulties (Fig. 1) and lower self-concept at 13 yrs were both associated with a higher BMI at 18 years. After adjusting for mediators, no statistically significant association was observed between ACEs and BMI (Fig 1). These results were similar for boys and girls.

Main Findings: The association between ACEs and BMI in adolescence that is mediated by behavioural difficulties and self-concept. A more complex model, such as a structural equation model, will need to confirm these findings.

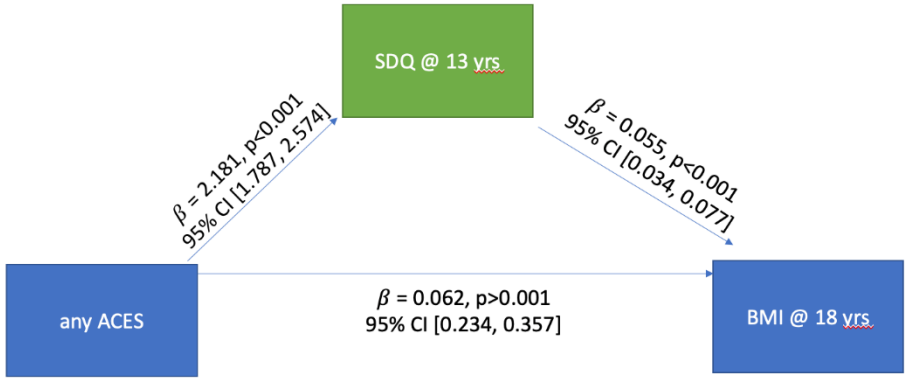

Figure 1. Model when adjusting for household income, household social class, primary caregiver BMI, diet, and exercise at 18 years.